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DETECTION OF FERMENTATIVE VARIANTS WITH TETRAZOLIUM[1]

JOSHUA LEDERBERG

Department of Genetics, University of Wisconsin, Madison, Wisconsin

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While trying to develop methods for finding dehydrogenase mutants, we found tetrazolium (triphenyl tetrazolium chloride) to be useful for detecting fermentative variants. This indicator has the advantage that the nonfermenting variants are stained a deep red whereas the fermenters and the background are neutral.

For example, cultures of *Escherichia coli* K-12 were spread on nutrient agar plates containing 1 per cent carbohydrate and 0.005 per cent tetrazolium. The plates were then irradiated with an ultraviolet lamp long enough to leave 100 to 500 colonies per plate after incubation. Red colonies or sectors were transferred to EMB agar to purify and verify them as mutants. Yields, ranging from 0.1 per cent to 0.01 per cent of the survivors, of mutants unable to ferment lactose, maltose, or glucose, respectively, have been obtained.

The response is undoubtedly due to the pH dependence of the biological reduction of tetrazolium to the highly colored, insoluble formazan. This usually accumulates in a single large granule near one pole of the cell.

Since *Salmonella* and *Shigella* cultures show a strong red reaction on lactose tetrazolium agar, this medium might be developed as an indicator for the enteric pathogens. However, it will be necessary to eliminate the reactions given by weak lactose fermenters (by controlling the pH and buffer capacity of the medium) and probably also to incorporate a selective inhibitor such as brilliant green.

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